



AFRL-AFOSR-JP-TR-2017-0057

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## Photosystem Inspired Peptide Hybrid Catalysts

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06/07/2017  
Final Report

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Air Force Research Laboratory  
AF Office Of Scientific Research (AFOSR)/ IOA  
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<b>REPORT DOCUMENTATION PAGE</b>					Form Approved OMB No. 0704-0188	
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<b>1. REPORT DATE (DD-MM-YYYY)</b> 10-08-2017		<b>2. REPORT TYPE</b> Final		<b>3. DATES COVERED (From - To)</b> 18 May 2015 to 17 Nov 2016		
<b>4. TITLE AND SUBTITLE</b> Photosystem Inspired Peptide Hybrid Catalysts				<b>5a. CONTRACT NUMBER</b>		
				<b>5b. GRANT NUMBER</b> FA2386-15-1-4019		
				<b>5c. PROGRAM ELEMENT NUMBER</b> 61102F		
<b>6. AUTHOR(S)</b> Ki Tae Nam				<b>5d. PROJECT NUMBER</b>		
				<b>5e. TASK NUMBER</b>		
				<b>5f. WORK UNIT NUMBER</b>		
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> SEOUL NATIONAL UNIVERSITY SNUR&DB FOUNDATION RESEARCH PARK CENTER SEOUL, 151742 KR				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>		
<b>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> AOARD UNIT 45002 APO AP 96338-5002				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b> AFRL/AFOSR IOA		
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b> AFRL-AFOSR-JP-TR-2017-0057		
<b>12. DISTRIBUTION/AVAILABILITY STATEMENT</b> A DISTRIBUTION UNLIMITED: PB Public Release						
<b>13. SUPPLEMENTARY NOTES</b>						
<b>14. ABSTRACT</b> <p>Biological systems can inspire to make new hybrid materials defined at the molecular level. We propose a novel way to make hybrid catalyst composed of inorganic nanomaterials and peptides. The specific peptides enable the assembly and synthesis of nanomaterials and enhance the catalytic activity synergistically. The focus of this proposed works is how to interface the peptide and inorganic materials for the hybrid materials. As a proof of concept, we aim to apply these new hybrid materials for oxidation reaction with the inspiration from the natural enzyme such as photosystem. Herein, we resolved fundamental questions related to protein/peptide folding structure and developed new bio-inspired organic-inorganic hybrid materials.</p>						
<b>15. SUBJECT TERMS</b> biomimetic, peptide						
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>  SAR	<b>18. NUMBER OF PAGES</b>  8	<b>19a. NAME OF RESPONSIBLE PERSON</b> AHLERS, KRISTOPHER	
<b>a. REPORT</b>  Unclassified	<b>b. ABSTRACT</b>  Unclassified	<b>c. THIS PAGE</b>  Unclassified			<b>19b. TELEPHONE NUMBER (Include area code)</b> 315-227-7009	

# **Final Report for AOARD Grant FA2386-15-1-4019**

## **“Photosystem Inspired Peptide Hybrid Catalysts”**

**05252017**

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**Period of Performance:** 05/18/2015 – 05/17/2016

### **Abstract**

Biological systems can inspire to make new hybrid materials defined at the molecular level. We propose a novel way to make hybrid catalyst composed of inorganic nanomaterials and peptides. The specific peptides enable the assembly and synthesis of nanomaterials and enhance the catalytic activity synergistically. The focus of this proposed works is how to interface the peptide and inorganic materials for the hybrid materials. As a proof of concept, we aim to apply these new hybrid materials for oxidation reaction with the inspiration from the natural enzyme such as photosystem. Herein, we resolved fundamental questions related to protein/peptide folding structure and developed new bio-inspired organic-inorganic hybrid materials.

### **Introduction**

In two-dimensional interfacial assembly, interplay between molecular ordering and interface geometry determines the final morphology and local geometry. We present the assembly of a designed peptide displaying facet formation of a water droplet. The identified tyrosine-rich peptides had a strong driving force to flatten the rounded top of droplet into a plane with a macroscopic 2D structure. The facet formation is driven by interactions of tyrosine and cross-linked stabilization by cysteine. We revealed the crucial sequence motifs for facet formation and their helical folding structures. The well-ordered redox active tyrosine in the 2D peptide film also can enhance chemical/electrochemical reaction.

In an effort to investigate the potential of tyrosine-rich peptide with redox active functions, we examine proton conductivity of the peptide films hybridized with manganese oxides. Proton conduction is an essential process to regulate the integral part of enzymatic catalysis and bioenergetics. To understand and manipulate proton conduction in bio-system, several studies have investigated bulk proton conduction of biomaterials. However, only little is known about that of short peptide-based materials. We focus on tyrosine-rich peptide having redox-active and crosslinkable phenol. The peptide/MnO<sub>x</sub> hybrid films can efficiently transport proton and proton conductivity was 18.6 mS cm<sup>-1</sup>. The value is much higher than that of biomaterials and comparable with synthetic materials. These results suggest that peptide-based hybrid material can be a promising new class of proton conductor.

Contrary to the conventional viewpoint on CO<sub>2</sub> as a major contributor of greenhouse gas, CO<sub>2</sub> also can be an abundant C<sub>1</sub> feedstock. Inspired by biological enzyme system, especially dehydrogenase catalyzing CO<sub>2</sub> reduction into formic acid, electrochemical CO<sub>2</sub> conversion, powered by renewable energy, has attracted considerable attention for achieving both objectives

of reducing carbon footprint and closing carbon-cycle. Although several metal-based electrodes such as Sn, Pb, Pd and In electrodes have been extensively investigated for CO<sub>2</sub> reduction catalysts, one of the most important challenges is to suppress the evolution of CO and H<sub>2</sub> in a much wider potential range than the current state. We first demonstrated that carbon-based BiO<sub>x</sub> hybrid material can selectively produce formate with a Faradaic efficiency (FE) more than 83% within an extremely wide potential window of approximately 0.5 V in 1.0 M NaHCO<sub>3</sub>/NaClO<sub>4</sub> solution

## **Experiment**

### **A. Dimerization process of monomeric peptide and facet formation**

The monomeric peptide was dissolved in deionized water. After the solutions rendered uniformly by tip sonicating, they was placed in a heat block at 80 °C for air oxidation. After incubation for hours the disulfide bonding (sulfur-sulfur bond) are formed from sulfhydryl residues of cysteine in monomeric peptides. After an extracted aliquot from the enough oxidized solution was dropped on siliconized glass, faceting started within a few minutes.

### **B. Two-dimensional nuclear magnetic resonance spectroscopy (2D NMR)**

All NMR spectra were measured at 298K on Agilent DD2 600 MHz spectrometer. For the assignments of amide and alpha protons, identification of sequential connectivity, and the secondary structure prediction of the peptide, 2D COSY, 2D TOCSY, and 2D NOESY experiments were performed. For TOCSY, an MLEV-17 spinlock pulse with a mixing time of 80 ms was used. For NOESY, the mixing times of 200 and 450 ms were used. All NMR spectra were processed with VnmrJ3.2 (Agilent Technologies, Inc.) and NMRpipe, and visualized with Sparky software. The 3 mM of peptide dissolved in 100% DMSO-d<sub>6</sub> was used for NMR experiments.

### **C. Electrical characterization of peptide/MnO<sub>x</sub> hybrid film devices**

The peptide/MnO<sub>x</sub> hybrid samples were characterized electrically in multiple distinct configurations. DC measurements on SiO<sub>2</sub>/Si substrates was conducted by Agilent 4156C semiconductor analyzer. AC measurements on quartz substrates was conducted by Agilent 4294A impedance analyzer. The humidity was monitored constantly with a hygrometer during all electrical experiments. The Nyquist plot was obtained using the frequency range from 100 Hz to 110 MHz with a constant applied voltage of 100 mV.

### **D. Evaluation of catalytic properties by electrochemical measurements and product analysis**

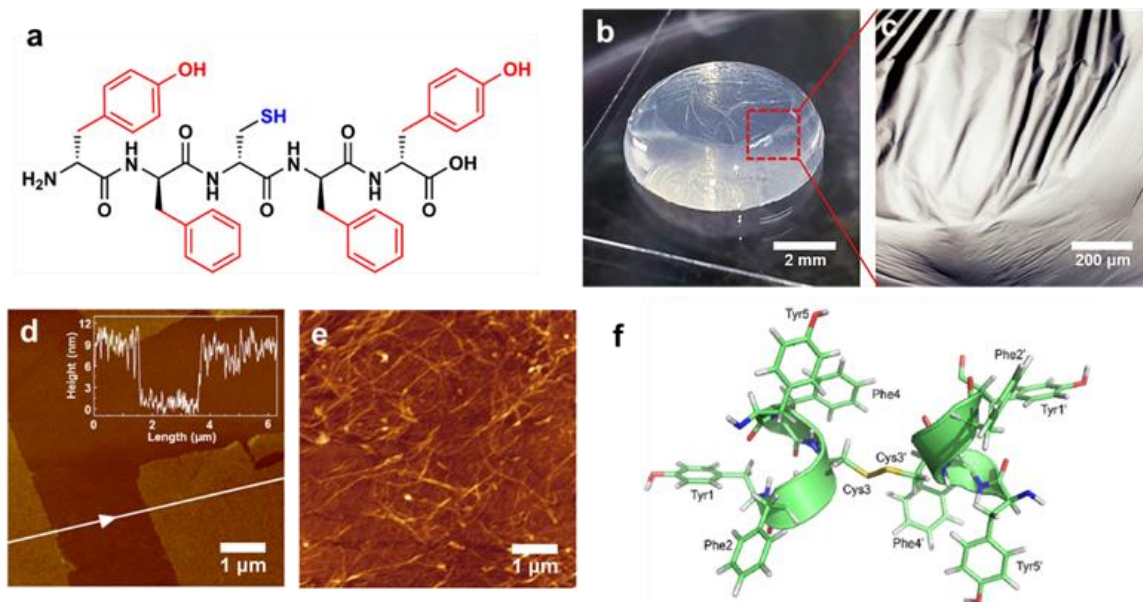
Electrolysis was conducted in a two-compartment electrochemical cell with a piece of Nafion membrane. The BiO<sub>x</sub>/C electrodes and a piece of platinum foil were used as working and counter electrodes, respectively. All potentials were controlled against an Ag/AgCl reference electrode (3.0 M NaCl, BASi) utilizing a potentiostat and converted to the RHE reference scale. Typically, before the electrolysis, the catholyte was saturated with CO<sub>2</sub> gas for at least 15 min to satisfy the saturation of pH. When the passed charge runs to 5 C, the electrolysis was stopped. Then, gas products were sampled using a syringe and injected to a gas chromatograph (Shimadzu) equipped with a packed Shincarbon ST 50/80 column. The gas mixtures in the column was transferred and detected in a thermal conductivity detector (TCD) and a flame ionization detector (FID). The concentration of produced HCOO<sup>-</sup> was analyzed by a 600 MHz NMR spectrometer (Avance 600, Bruker). The catholyte containing

the liquid product was mixed with the D<sub>2</sub>O solution of DMSO and phenol as internal standards.

## **Results and Discussion**

### **A. Identification of major determinant for the assembly in tyrosine-based peptide and its folding structure (Figure 1)**

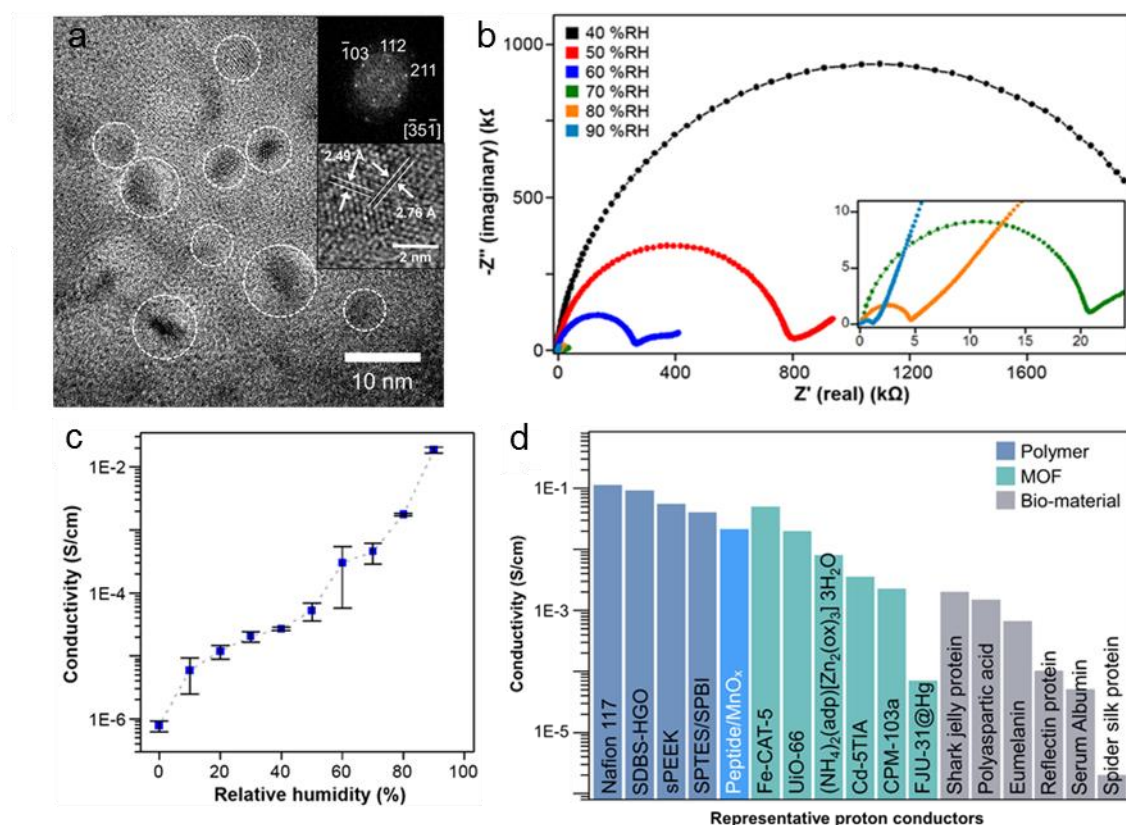
Enzymes in nature transport electrons using the appropriate folding structure of the active sites of the metal ion surrounding peptides. In order to fabricate an enzyme-like materials using peptides, it is very important to increase the conductivity of the peptide. Understanding and applying the folding structure of peptides is critical to achieving the purpose. Based on tyrosine (Y) amino acid, we found the peptide sequence (YYACAYY) which has electrochemical properties essential for self-assembly of a large-area film with a thickness of several tens of centimeters while retaining the folding and functionality of the peptide. Sequence. Based on this technology, we succeeded in expanding the peptide sequence capable of large self-assembling by introducing phenylalanine (F) to enhance pi-pi stacking force. After constructing peptide sequence library, we found that YFCFY sequence can also assemble into macroscopic 2D nanosheets. As a result of measuring the time of formation of the film by adjusting the ratio of the dimer to the monomer, it was found that the higher the dimer ratio, the shorter the formation time. From the above results, it has been found that a stable dimer structure should be formed through a disulfide bridge in order to self-assemble into a large-area film structure. Furthermore, this self-assembled structure was analyzed by 2D-NMR and circular dichroism, and it was found that the helical structure was arranged in the dimer form.



### **B. Synthesis of proton conducting peptide/metal oxide hybrid materials (Figure 2)**

Tyrosine is known to be an important sequence that helps electron transfer and hydrogen ion transfer in the natural world. In particular, for enzymes containing metal ions containing metal ions, it is found that most of the tyrosine is located adjacent to the metal ion. The phenol group of tyrosine facilitates the transfer of electrons and hydrogen ions, which are essential when the enzymatic action takes place. Tyrosine is also a precursor of melanin,

which is also known as a natural semiconductor polymer. As the tyrosine undergoes oxidation, it polymerizes and eventually turns into melanin. Utilizing the characteristics of tyrosine, the team formed a film with a YYACAYY sequence rich in tyrosine, and then treated with  $\text{KMnO}_4$ , an oxidant, to induce the oxidation/polymerization of tyrosine and to form a hybrid structure with manganese oxide). The peptide/manganese oxide film was produced by a simple method without high temperature heat treatment or toxic chemical composition. By measuring the electrical behavior of the hybrid material with electrochemical impedance spectroscopy, it was found that the hybrid material showed hydration dependent electrical behaviors. The peptide/ $\text{MnO}_x$  hybrid film can efficiently transport proton at humid condition and its proton conductivity was  $18.6 \text{ mS cm}^{-1}$ . Such high proton conductivity for a peptide/ $\text{MnO}_x$  hybrid material is unprecedented even though it is reported that certain phases of manganese oxides and peptide/protein containing a large amount of carboxylic groups or hydrophilic moieties exhibit moderate proton conduction at room temperature. Comparison with literature data on low-temperature proton conductors showed that the conductivity of the peptide/ $\text{MnO}_x$  hybrid film is the highest among biomaterial-based proton conductors. The value is comparable to that of metal-organic frameworks, and it is only an order of magnitude lower than the proton conductivity of Nafion, the state-of-the-art proton conductor.

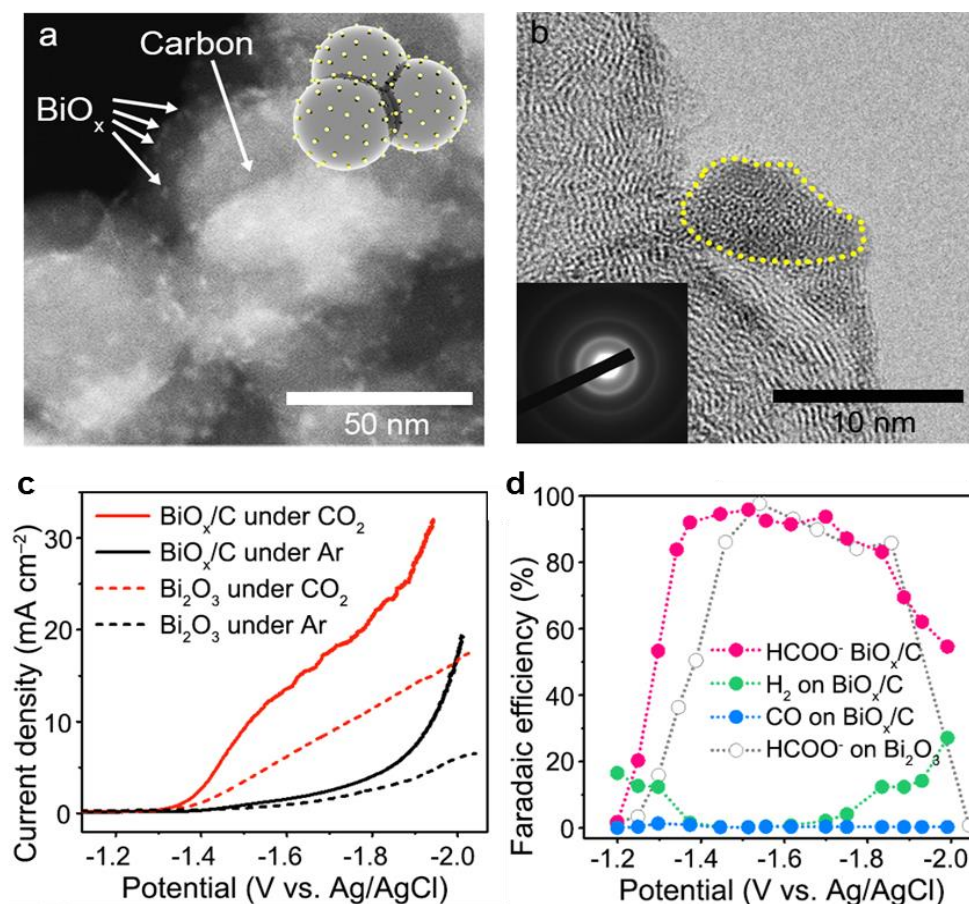


### C. Synthesis of enzyme-mimetic electrochemical catalysts (Figure 3)

Global carbon dioxide levels in the atmosphere have surpassed 400 ppm for the first time in recorded history. Electrochemically converting  $\text{CO}_2$  into useful chemicals and fuels represents a promising strategy to not only reduce  $\text{CO}_2$  emission, but also replace or modify current petrochemical-based processes. Among  $\text{CO}_2$ -derived fuels, formic acid ( $\text{HCOOH}$ ) is the most attractive candidate as a liquid fuel for the hydrogen economy due to its high



volumetric hydrogen density of 53 g H<sub>2</sub> per liter, non-toxicity, safety and transportability. The rapid, reversible, and specific electrochemical reduction of CO<sub>2</sub> to formate by a formate dehydrogenase from the anaerobic bacterium *Syntrophobacter fumaroxidans* provided the paradigm case for a formate/CO<sub>2</sub> catalyst. The dehydrogenase catalyzes the rapid interconversion of CO<sub>2</sub> and formate at the reduction potential for the reaction. Inspired by this enzymatic CO<sub>2</sub> reduction chemistry, we developed efficient and environmentally benign catalysts for formate conversion with carbon-based bismuth oxide. Figure 3a and the schematic representation show that white Bi oxide nanoparticles with sizes of < 10 nm could be clearly distinguished from the gray carbon particles with sizes of several tens of nanometers. The analyses of HRTEM and SAED patterns showed that the synthesized bismuth oxide was amorphous, which was also identified by XRD analysis. The CO<sub>2</sub> reduction capability of the BiO<sub>x</sub>/C catalyst was demonstrated by linear sweep voltammetry. As shown in Figure 2a, the BiO<sub>x</sub>/C electrode under CO<sub>2</sub> exhibited enhanced catalytic current compared to that under Ar. At -1.41 V vs. Ag/AgCl, the current density reached over 3.5 mA cm<sup>-2</sup> under CO<sub>2</sub>, a value that was 4.6-fold larger than that observed under Ar. To quantitatively analyze the reaction products and associated faraday efficiencies (FEs) of BiO<sub>x</sub>/C, bulk electrolysis was conducted at fixed potentials. The FE for formate reached 92.1% at -1.37 V vs. Ag/AgCl and was stably maintained at an average value of 93.4% until the potential reached -1.70 V vs. Ag/AgCl. At -1.75 V vs. Ag/AgCl, the FE for formate declined, and the FE for H<sub>2</sub> gradually increased from 4.1% to 27.2%. Conversely, near the onset potential for formate formation, the FE for H<sub>2</sub> decreased from 16.6% to 1.6%. In contrast to the competing trend between H<sub>2</sub> and formate, the CO evolution by BiO<sub>x</sub>/C was largely suppressed over the whole potential range.



**List of Publications and Significant Collaborations that resulted from your AOARD supported project:**

- a. Chan Woo Lee, Jung Sug Hong, Ki Dong Yang, Kyoungsuk Jin, Jun Ho Lee, Hyo-Yong Ahn, and Ki Tae Nam, Selective Electrochemical Production of Formate from Carbon Dioxide with Bismuth-Based Catalysts in an Aqueous Electrolyte, *Acs Catalysis*, accepted

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